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# A CHROMOGENIC BACILLUS FROM A CASE OF ROUP

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A buff plymouth rock cock, 2 years old and a member of the station and college flock, was apparently in good health till about 3 weeks previous to his death. The bird became dull, thinner in flesh, the comb dark and somewhat shriveled and there was difficult breathing and a general unkempt appearance. On opening the mouth there was noted a mass of diphtheric material formed from a wide-spread oral diphtheric area. The nasal passages were partially occluded with a thin watery discharge. Breathing was partially oral.

At the necropsy the following observations were made: There are no evidences of disease except in the region of the nostrils and mouth. There is an offensive roupy odor from the mouth. In the mouth cavity is observed a wide-spread ulcerated area covered with a yellowish-white membrane, diphtheric in nature. There is a mucopurulent accumulation in the nasal passages and in the trachea.

Microscopically the kidneys show both active and passive congestion. The cells of the tubules are in a state of cloudy swelling. Many nuclei of the cells of the convoluted tubules are in a state of karyorrhexis, other cells show a total disappearance of the nuclei. The lumen of many of the convoluted tubules are obliterated and the cell bases have moved in from their peripheral positions.

Smears from the lower third of the trachea showed apparently almost a pure culture of a rather short thick organism about two to three times as long as broad. From the purulent tracheal material, made in suspension in salt solution, there was inoculated intraperitoneally into a half-grown rabbit, 4 c c. The rabbit died of septicemia in 20 hours. Pure cultures of an organism, which has been designated as 3a, were isolated. These pure cultures were obtained from the heart blood, liver, and peritoneal fluid.

A second rabbit was inoculated with 1 c c of an emulsion made from the culture of the heart blood of Rabbit 1. In preparing the emulsion just as much culture was used as would adhere to one-fourth inch space of the platinum needle. This injection was made intraperitoneally. The rabbit died of septicemia in 18 hours. Pure cultures were obtained from the heart blood, liver, and the peritoneal fluid.

*Character of Organism 3a.*—Bacillus on artificial medium. Rather coccoid in form from heart blood of animal which has died of septicemia from this organism. In later transfers the organism is distinctly bacillary in form.

Cultures 5 days old show organisms averaging 0.49 by 2.3 microns and from the heart blood of the rabbit 0.43 by 0.64 microns.

Ends rounded.
No spore formation.
Flagella, peritrichiate.
Capsule, absent.
Growth at 37 C., abundant.

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Staining reactions, readily with ordinary anilin dyes as fuchsin, methylene blue, gentian violet, Loeffler's methylene blue.

Reaction to Gram stain, negative.

*Cultural Features.*—Agar stroke, growth, moderate. Form of growth, echinulate. Elevation, raised. Luster, glistening. Topography, surface smooth, porcelain-like. Optical characters, opaque. Chromogenesis, green, varying from a turtle green at the end of 2 days to a shamrock green in old cultures. Comparisons made with Ridgway's color chart.

*Potato.*—Growth, scanty. Form of growth, spreading. Elevation, flat. Luster, dull. Topography, contoured. Chromogenesis, brownish with a tendency to green later. Odor, decided. Consistency, butyrous. Medium, browned. Old cultures, brownish-green. The diastatic action was feeble.

*Agar Stab.*—Growth, abundant. Best on top. Surface growth, abundant. Line of puncture, filiform.

*Gelatin Stab.*—Growth, best on top. Line of puncture, filiform. Liquefaction, stratiform. Medium, greened.

*Nutrient Broth.*—Surface growth, skumlike. At times scanty with pellicle. Clouding, at first moderate, later strong. Odor, decided. Sediment, granular—on agitation abundant.

*Milk.*—Clearing without coagulation. Digested medium gradually becomes greenish.

*Agar Colonies.*—Dew drop colonies. Colonies at the end of 24 hours are smooth but in older colonies the culture has a tendency to become grumous.

*Growth in Broth Over Chloroform.*—At first feeble, but after a few days there had developed a moderate growth. Chloroform restrains growth.

*Growth in Glycerol Broth.*—The organism grew abundantly but without gas or acid formation.

*Reduction of Nitrates.*—At the end of 14 days there was a marked reduction of nitrates.

*Toleration of Acids.*—Fuller's scale:

| Acid | 24 hours | 48 hours | 72 hours | 7 days |
|---|---|---|---|---|
| + 10* | + | + | + | + |
| + 20 | — | — | — | — |
| + 30 | — | — | — | — |
| + 40 | — | — | — | — |
| + 50 | — | — | — | — |

* Control tube.

There was no growth in any of the tubes. The control showed abundant growth.

*Toleration to NaOH.*—Fuller's scale:

| NaOH | 24 hours | 48 hours | 72 hours | 7 days |
|---|---|---|---|---|
| — 10* | + | + | + | + |
| 0 | + | + | + | + |
| + 10 | + | + | + | + |
| + 20 | — | + | + | + |
| + 30 | — | — | + | + |
| + 40 | — | — | — | + |
| + 50 | — | — | — | — |
| + 60 | — | — | — | — |

* Control tube.

Plus 50 completely arrested growth; 40 showed slight growth at the end of 7 days, and 30 at the end of 72 hours.

The vitality of the culture is long continued on artificial medium. The optimum temperature for growth is 37 C.

*Test Against Phenol.*

| Percentage in tube | 24 hours | 48 hours | 72 hours | 96 hours | 7 days |
|---|---|---|---|---|---|
| control | + | + | + | + | + |
| 0.1 | + | + | + | + | + |
| 0.25 | — | + | + | + | + |
| 0.5 | — | — | — | — | — |
| 0.75 | — | — | — | — | — |
| 1.0 | — | — | — | — | — |
| 2.0 | — | — | — | — | — |
| 3.0 | — | — | — | — | — |
| 4.0 | — | — | — | — | — |
| 5.0 | — | — | — | — | — |

Phenol, 0.25%, arrested growth and 0.5% killed the organisms.

*Thermal Death Point.*—Time of exposure in water bath, 10 minutes.

| Temperature, C. | 24 hours | 48 hours | 72 hours | 96 hours | 7 days |
|---|---|---|---|---|---|
| control | + | + | + | + | + |
| 50 | + | + | + | + | + |
| 55 | — | + | + | + | + |
| 60 | — | — | — | — | — |
| 65 | — | — | — | — | — |
| 70 | — | — | — | — | — |
| 75 | — | — | — | — | — |

The exposure to 55 C. arrests growth and 60 C. kills the organisms.

Test tubes containing 10 c c each of broth were inoculated with Culture 3a and the surface covered with autoclaved oil of petrol. The results were negative though the cultures were incubated 10 days.

Below is given the results of the action of this organism on 6 different sugars. All fermentation tubes contained 1% of the sugar. Titrations made at the end of 14 days.

| Sugar | Control | Tube 1 | Tube 2 |
|---|---|---|---|
| Saccharose | +1 | +0.5 | +0.5 |
| Lactose | +1 | +0.5 | +0.5 |
| Dextrose | +1 | +1.0 | +1.0 |
| Maltose | +1 | +0.5 | +0.5 |
| Raffinose | +1 | +0.5 | +0.5 |
| Mannite | +1 | +1.0 | +1.0 |

There was no change in the dextrose and mannite. There was slight alkali formation in the saccharose, lactose, maltose and raffinose.

*Pathogenicity.*—In the early part of the paper the pathogenicity to rabbits has been shown. Several birds were inoculated. The following average result will serve to show the effect on the fowl. Inoculations were made in the infraorbital sinus, nostrils, skin of the face, intratracheally, comb, wattles, mouth, and eye. The surface inoculations were made intradermally. At the end of 24 hours there was noted at the point of inoculation, of the comb and wattles, considerable swelling. In the mouth there was a white ulceration of the mucous membrane at the point of inoculation. This ulcer showed little tendency to spread and healed by the end of the 5th day. The tumefaction of the comb attained the size of a half corn grain, some ulcerating and others subsiding after a few days without ulceration. Some birds showed more resistance than others. Half-grown single comb white leghorn cockerels were used to ensure none having gained artificial immunity, as in this section most flocks, sooner or later, go through at least a mild attack of chickenpox or roup. In those birds in which ulcers developed there was present depression, weakness, partial loss of appetite, lopped comb and a rapid loss of weight. In those cockerels in which ulceration did not take place and in which the swelling soon subsided these symptoms were not noticed. In those cases in which ulceration took place recovery occurred in from 10-14 days. The nasal, intratracheal and infraorbital inoculations on the uninjured mucous membranes were all negative.

## DISCUSSION

In the study of roup at this station there has been isolated an organism which, so far as our literature goes, perhaps has not hitherto been described. The organism is a rather short, thin bacillus measuring, on an average, 0.43 by 0.64 mikrons. The ends are rounded. It is peritrichiate, hence motile. It does not possess capsule. It grows abundantly at 37 C. and produces a greenish pigment. It is gram-negative and stains readily with ordinary anilin dyes. It is aerobic, growing very little along the needle track and when the oxygen is entirely excluded it does not grow. It reduces nitrates; 2% normal HCl completely arrests its development; 2% NaOH arrests but does not completely retard its growth as at the end of 48 hours feeble development was noted in all tubes with this percentage; 5% completely arrested its growth; 0.25% phenol retarded its growth and 0.5% killed it; 55 C. retarded its growth and 60 C. killed it.

There was no change in dextrose and mannite broth nor any gas formation. There was a slight formation of alkali in saccharose, lactose, maltose and raffinose. There was no gas formation.

The organism is highly pathogenic in rabbits, killing of septicemia in 18-20 hours, and shows some pathogenic properties for the fowl when injected into injured tissues as in intradermal inoculations.

In terms of the numerical system of recording the salient characters of an organism (group number), as suggested by the Society of American Bacteriologists, we have as a total 211.3331423.

## SUMMARY

There is here given an account of a chromogenic bacillus found in connection with roup and chickenpox probably not before reported.

The organism described is highly pathogenic for rabbits and shows some pathogenic properties for the tissues of the fowl.